The quantitative results obtained from the occipital hair were not consistent with those of the vertex hair (Supplementary Table S2 and S3 online). The occipital T levels were not significantly different between the Korean groups, a finding that is not concordant with that of an earlier report obtained from vertex hair (Choi *et al.*, 2001). In addition, the DHT/T ratio, one of the indicators of MPB (Choi *et al.*, 2001; Bang *et al.*, 2004), was lower in the vertex hair after treatment with 5α-reductase inhibitors, both finasteride and dutasteride, but not in the occipital hair (Ryu *et al.*, 2006; Jung *et al.*, 2011). All vertex steroid levels except for 11β-OH-An tended to be slightly lower than occipital steroid levels in individual hairs. No significant differences between two sites were found in any case. This finding suggests that the distribution of androgens differs for each region of the scalp and may be useful for intersite comparison (Rushton *et al.*, 1991).

The differences in the metabolic ratio of DHT to T obtained from the vertex hair shafts were not statistically significant in any of the groups (Supplementary Table S1 online). An alternative metabolic ratio responsible for 5α-reductase activity with A-dione and T was therefore introduced because A-dione and T are reversibly catalyzed by 17β-HSD in the androgen metabolic process. Although these metabolic ratios tended to increase in both Caucasian groups, the differences were not statistically significant (Supplementary Table S1 online). In contrast to the 5α-reductase activity, the activity of 3β-HSD, which is indicated by the A-dione to DHEA metabolic ratio, was higher in the Korean groups. At the target cell levels, different androgenic productions could be regulated by the balance between 5α-reductase, 3β-HSD, and 17β-HSD (Eicheler *et al.*, 1998).

Although our findings are based on the epithelial environments and not on the papilla cells, it confirms the existence of racial differences in hair steroid levels, and the results highlight the necessity for careful monitoring and controlling for multiple factors along with race before making a conclusion with result from the biological fluids. Further studies would need to compare the drug efficacies in the balding groups to provide a personalized and evidence-based approach to patient treatment.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Man Ho Choi[1,2], Sun Ju Kim[1,2], Bark-Lynn Lew[3], Woo Young Sim[3] and Bong Chul Chung[1,2]

[1]Future Convergence Research Division, Korea Institute of Science and Technology, Seoul, Korea; [2]Department of Biomolecular Science, University of Science and Technology, Daejeon, Korea and [3]Department of Dermatology, Kyung Hee University, Seoul, Korea
E-mail: bcc0319@kist.re.kr

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Mosaic Activating *RAS* Mutations in Nevus Sebaceus and Nevus Sebaceus Syndrome

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TO THE EDITOR

Nevus sebaceus is a common congenital skin hamartoma, classically appearing as a yellow-hued plaque on the scalp, face, or neck. It is the hallmark lesion of Schimmelpenning/nevus sebaceus syndrome (OMIM: 163200), a multisystem disorder that includes a spectrum of central nervous system, ocular, skeletal, and cardiovascular defects. Secondary neoplasms arise within nevus sebaceus at a modest but elevated rate (Moody *et al.*, 2012), prompting disagreement about whether they should be routinely excised (Shwayder, 2011). Determining the pathogenesis of nevus sebaceus would provide a framework to better understand this lesion and its associated syndrome.

The appearance of nevus sebaceus along Blaschko's lines suggests that a

Abbreviations: MAPK, mitogen-activated protein kinase; pERK, phosphorylated ERK

mosaic genetic mutation causes the lesion, with more extensive multisystem involvement potentially underlying the syndromic form (Happle, 1993). Here, we report a case of an individual with nevus sebaceus syndrome. As individuals with this syndrome are uncommon, we sought to identify associated mutations by comparing the exome sequence of the nevus sebaceus from our patient with those of sporadic nevus sebaceus.

Our index patient is a 38-year-old woman who was born with Chiari malformation, myelomeningocele, and resultant paraplegia. Because of hydrocephalus and marked ventricomegaly, she required ventriculo-peritoneal shunt placement. Imaging studies demonstrated rotoscoliosis. She has had cognitive developmental delay and suffered from generalized seizures during childhood. In her early thirties, she experienced a middle right cerebral artery stroke. Despite her condition, she remains high functioning and lives in an assisted care facility. No other family members are affected by similar medical conditions, and no cause has been attributed to her findings.

In the past year, the patient became bothered by growths on her forehead and presented to our clinic. On examination, frontal bossing was observed, as well as a yellow-hued papillomatous plaque on the paramidline forehead that had been present since birth (Figure 1a). Several pedunculated papules were noted within the lesion. No other significant cutaneous findings were appreciated.

As per patient request, the lesion was excised and a portion of the excision specimen was collected with her written informed consent. Our study complied with the Declaration of Helsinki Principles and was approved by the Stanford Institutional Review Board. Histological evaluation confirmed features of nevus sebaceus with no secondary neoplasms (Figure 1b). Accordingly, in light of the extensive neurological and skeletal involvement, a diagnosis of Schimmelpenning/nevus sebaceus syndrome was made. In efforts to determine an underlying genetic mutation, four additional, independent nevus sebaceus samples were collected from elective excisions along with adjacent normal skin controls. The five samples were subjected to exome sequencing and analyzed for mutations using Seqgene (Deng, 2011) and DNAnexus (http://www.dnanexus.com) as described in the Supplementary Material online.

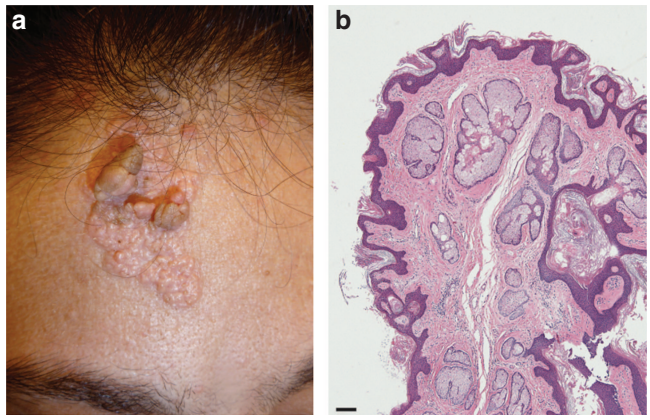

Figure 1. Clinical and histological features of a patient with nevus sebaceus syndrome. (**a**) A yellow-hued, papillomatous, oblong plaque on the paramidline forehead of the index patient. (**b**) Hematoxylin and eosin–stained section (original magnification ×40) of a pedunculated papule from the patient's lesion showing epidermal acanthosis, papillomatosis, the absence of hair follicles, and ectopic sebaceous glands opening directly to the epidermal surface. Bar = 100 μm.

Analysis of recurrent lesion-specific variants identified an *HRAS* point mutation (c.37G>C, p.Gly13Arg) in the index case and in two of four isolated nevus sebaceus samples, with a variant allele frequency ranging from 17 to 43%. Sanger sequencing confirmed the *HRAS* mutation in all five lesional samples and its absence in all matched controls (Figure 2a). Examination of the two *HRAS* mutation–negative exomes showed low sequence coverage (<20 reads) at the mutation site, which may account for the false-negative calls.

Lesions arising along Blaschko's lines are hypothesized to stem from a mosaic mutation affecting a specific cell lineage during development. To evaluate whether the candidate mutation fits this criterion, we used laser capture microdissection to isolate DNA from the lesional epidermis and dermis from the index case and one of the sporadic nevus sebaceus samples. In both cases, the mutation was limited to the epidermis, supporting the hypothesis of an acquired mutation affecting ectodermal precursors (Figure 2b). Both alleles were represented in approximately equal intensities, indicating that the mutation is likely heterozygous.

We next performed targeted Sanger sequencing on a validation set of 31 independent nevus sebaceus samples from archived tissues, and identified the *HRAS* p.Gly13Arg mutation in 24/31 samples and p.Gly12Asp in one sample. The remaining mutation-negative cases were evaluated for *KRAS* and *NRAS* hotspot mutations, identifying two samples carrying *KRAS* p.Gly12Asp mutations. Six validation samples had patient-matched normal skin tissue available, and the corresponding *RAS* mutations were absent in all six control samples. In total, 32 of 36 samples (89%) demonstrated *HRAS* or *KRAS* mutations, confirming a strong correlation between activating *RAS* mutations and nevus sebaceus (Figure 2c). We suspect that the remaining negative cases may be due to genetic heterogeneity, or due to a low mutant allele frequency secondary to admixture with normal tissue.

RAS promotes cell growth through activation of multiple pathways, a main pathway being the mitogen-activated protein kinase (MAPK) signal-transduction pathway. Activating mutations in this gene family have well-established links to cancer (Schubbert *et al.*, 2007). Germline activating *HRAS* mutations cause Costello syndrome, which features predisposition to neoplasia and development of cutaneous papillomas (Gripp and Lin, 2012). Taken together, the known biological features of activated *RAS* genes are consistent with the hamartomatous

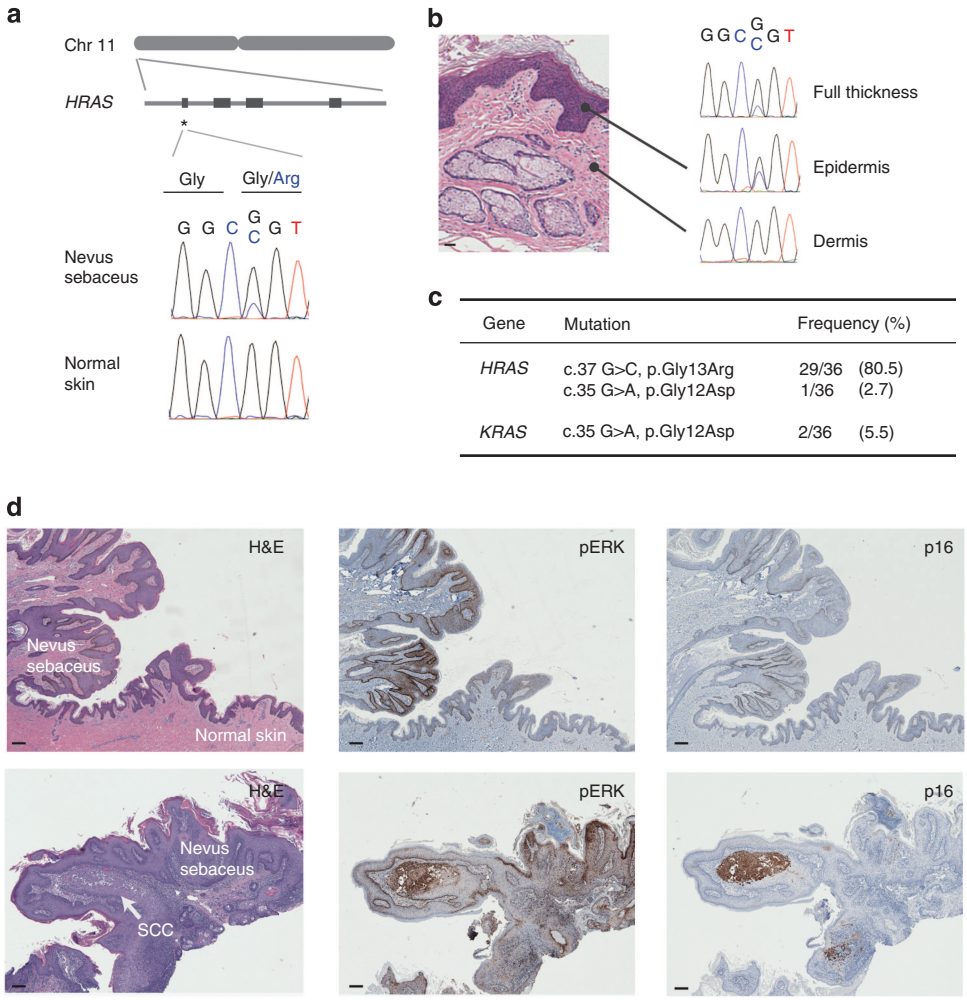

Figure 2. Activating mosaic *RAS* mutations in nevus sebaceus. (**a**) Genomic localization of *HRAS* to the short arm of chromosome 11 and schematic of its gene structure. A prominent mutational hotspot in coding exon 1 (codons 12–13) is marked with an asterisk. Sanger sequencing confirms a c.37 G>C, p.Gly13Arg mutation specific to lesional tissue. (**b**) Laser capture microdissection of the epidermis and dermis of nevus sebaceus demonstrates the presence of the *HRAS* mutation exclusively in the epidermis. (**c**) Summary of *RAS* mutations identified in nevus sebaceus. (**d**) The activated RAS/mitogen-activated protein kinase (MAPK) pathway in nevus sebaceus. Upper row: nevus sebaceus transitioning into normal skin. Immunohistochemical staining for phosphorylated ERK (pERK), a downstream effector of the *RAS* pathway, is increased in nevus sebaceus compared with adjacent normal skin. Staining for p16 is homogenously negative. Lower row: an early focus of squamous cell carcinoma (SCC) arising within a nevus sebaceus. This area is characterized by stronger pERK signal and distinct p16 enrichment. All original magnifications are at ×40. Bar = 100 μm. H&E, hematoxylin and eosin.

overgrowth and elevated neoplasia risk observed in nevus sebaceus.

To evaluate *RAS*-MAPK signaling, we performed phosphorylated ERK (pERK) staining on a set of nevi with confirmed *HRAS* mutations. Immunohistochemistry revealed increased pERK staining in lesional versus normal epidermis, consistent with *RAS*-MAPK hyperactivation (Figure 2d). In one sample, a squamous cell carcinoma was identified arising from nevus sebaceus, highlighted by elevated p16 staining (Hodges and Smoller, 2002). The pattern of neoplasia arising from a background of upregulated pERK supports the hypothesis that *RAS*-MAPK hyperactivation may predispose toward the development of secondary neoplasms in nevus sebaceus.

Basal cell carcinomas were once thought to arise commonly from nevus sebaceus, but others have subsequently contended that the majority of these tumors are actually trichoblastomas (Cribier *et al.*, 2000). Our data provide genetic support for the latter opinion, as most basal cell carcinomas arise from Hedgehog pathway dysregulation and lack *RAS* mutations (Reifenberger *et al.*, 2005). Our findings also raise the possibility that tumors arising from nevus sebaceus, such as syringocystadenoma papilliferum and trichoblastomas, may be associated with *RAS* mutations as well.

Using targeted sequencing and SNaPshot assays, Groesser *et al.* (2012) and Hafner *et al.* (2012) have recently profiled oncogenic hotspot mutations in epidermal and sebaceus nevi. Together with the results presented here and by others in this issue (Levinsohn *et al.*, 2013), the cumulative data demonstrate that keratinocytic epidermal nevi and sebaceus nevi are both associated with activating *HRAS* p.Gly13Arg and *KRAS* p.Gly12Asp mutations, supporting the belief held by some clinicians that they represent a spectrum of the same entity

(Sybert, 2010). We postulate that the phenotypic difference between these nevi may be related to the extent of the mutation, as well as body site–specific embryological patterns and environment. The knowledge of the genetic basis of nevus sebaceus and its associated syndrome represents a further step toward understanding genotype–phenotype correlations arising from genetic mosaicism.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Bryan K. Sun[1], Andrea Saggini[2,3], Kavita Y. Sarin[1], Jinah Kim[1], Latanya Benjamin[1], Philip E. LeBoit[2] and Paul A. Khavari[1]

[1]Department of Dermatology, Stanford University School of Medicine, Stanford, California, USA; [2]Department of Dermatology, University of California, San Francisco, San Francisco, California, USA and [3]Department of Dermatology, University of Rome Tor Vergata, Rome, Italy
E-mail: bryansun@stanford.edu

SUPPLEMENTARY MATERIAL

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Whole-Exome Sequencing Reveals Somatic Mutations in *HRAS* and *KRAS*, which Cause Nevus Sebaceus

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TO THE EDITOR

Epidermal genetic mosaicism is evident as stripes of affected skin that typically appear in S- or V-shaped whorled, streaked, and linear patterns called lines of Blaschko (Blaschko, 1901). These patterns represent dorsoventral migratory pathways of neuroectoderm during embryogenesis (Moss *et al.*, 1993). Mosaic lesions result from somatic mutation during development, with timing of such events determining the extent and distribution of skin involvement. Epidermal nevi (EN) are common cutaneous mosaic disorders seen in 0.1–0.3% of births, and fall into two classes: keratinocytic epidermal nevi (KEN) and organoid epidermal nevi, which includes nevus sebaceus (NS) and follicular nevi (Solomon and Esterly, 1975). NS comprises approximately half of EN, and typically appears on the scalp as a yellowish–orange linear plaque with hyperkeratosis, acanthosis, a markedly increased number of sebaceous lobules, and abortive hair follicles with resulting alopecia (Figure 1a–d). In contrast to KEN, in which neoplasia is rare, tumors develop in nearly 14% of all NS cases, and in more than 23% of affected adults (Cribier *et al.*, 2000), suggesting that the mutation(s) causing NS also increase the risk of tumorigenesis (Figure 1e–g).

Recently, somatic mosaicism has been identified in KEN using SNaPshot assays to identify mutations in mitogen-activated protein kinase (MAPK) pathway genes including *FGFR3*, *HRAS*, *KRAS*, *NRAS*, and *PIK3CA*. Activating Ras mutations, including *HRAS* p.Gly13Arg and *KRAS* p.Gly12Asp, were most common and accounted for 39% of KEN, with *HRAS* mutations predominating (Hafner *et al.*, 2012). Similar approaches have been used in NS, identifying *HRAS* p.Gly13Arg in 91% of lesions and *KRAS* p.Gly12Asp in 5% of lesions (Groesser *et al.*, 2012). We present an independent, complementary approach to genetic pathogenesis in NS, in which we used whole-exome sequencing to characterize the spectrum of *de novo* coding mutations present within NS lesions.

Abbreviations: EN, epidermal nevi; KEN, keratinocytic epidermal nevi; LOH, loss of heterozygosity; MAPK, mitogen-activated protein kinase; NS, nevus sebaceus